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IJPBS |Volume 4| Issue 2|APR-JUN|2014|109-116

Research Article
Biological Sciences

BIOREMEDIATION OF TEXTILE DYE EFFLUENT BY *SHEWANELLA PUTREFACIENS*

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ABSTRACT

The aim of the present study was to use a bacterial isolate for decolorization and reduction of pollution from textile effluent. Shewanella putrefaciens was isolated from raw textile effluent and used to decolorize the effluent, in unoptimized and optimized conditions. In unoptimized condition the decolorization was 27.95% whereas in optimized condition it reached 63.15% (Chrysophenine optimized value) and 89.4% (Red 3BN optimized value). It was essential to substitute the raw effluent with co-factors to enhance decolorization process. It was also important to provide suitable culture conditions to improve the decolorization efficiency, in the absence of which the bacterial isolate could not decolorize to the extent required. The physicochemical characterization of the effluent was carried out before and after decolorization process to assess the change in the parameters and thereby the pollution load on the environment. Sulphate (79.4%) and Phosphate (78.6%) showed highest reduction whereas Total suspended solids, BOD and COD showed 55%, 41.1% and 30.4% reduction, respectively. This study recommends the application of shewanella putrefaciens, as a potent bacterial strain in decolorization of textile dyes and effluents under suitable nutritional and environmental conditions.

KEY WORDS

Effluent decolorization, Optimized and unoptimized culture condition, Physico-chemical characterization, Shewanella putrefaciens

INTRODUCTION

Textile industries consume large quantities of water and generate huge amounts of effluents containing considerable amounts of suspended solids, additives, detergents, surfactants, carcinogenic amines, aldehydes, heavy metals and dyes. Fluctuating pH, high temperature, COD, BOD and complex coloration are main physico-chemical characters of textile effluents and as such they pose serious environmental threats to receiving water bodies [1]. Color is the first contaminant to be recognised in textile effluent as it is the major contributor of biological oxygen demand [2]. The discharge of highly colored effluents containing dyes can be damaging to the receiving bodies and can result in serious environmental pollution problems. These textile effluents have common characteristics due to high coloration, since even a small amount of residual dye (mg/l) can cause a significant visual effect and affect the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies.

Among the commonly used dyes, azo dyes are difficult to decolorize because of their complex structure and synthetic origin. Biodegradation of azo dyes is possible if the azo bond is first reduced. Permanent decolorization of azo compounds occurs on cleavage of azo bond but the intermediates can be reoxidised to colored by-products. Majority of these dyes are toxic, mutagenic and stable to light and temperature. These properties inhibit attack by microorganisms. Removal of such dyes is a matter of serious concern [3].

The reuse of industrial effluents can be an attractive option for meeting the increasing demand for water.

For this purpose, biological method of using bacteria has been shown to be efficient, eco-friendly and more cost-effective [4]. The bacteria show very promising ability to decolorize, degrade, detoxify and metabolize a number of compounds in various biological treatment processes. Due to ubiquitous nature of bacteria, they can be used as invaluable tools for the biological treatment of textile effluent. As a preliminary step in the development of biological treatment of textile effluent, there is a need to isolate more bacterial strains having potential to decolorize and degrade textile dyes and remove other pollution parameters [5].

Therefore, in the present study attempt has been made to explore the potential of a bacterial strain, *Shewanella putrefaciens* to decolorize the textile dye effluent in optimized and unoptimized conditions. Assessment has also been made of the variation in physico-chemical parameters of the raw and the treated effluent before and after decolorization process.

MATERIALS AND METHODS

Effluent

The effluent sample was collected from the textile industry located in Nanjangud, Karnataka using polythene container. The effluent was filtered to remove the floating materials and sterilized to kill all the microorganisms. After the pre processing, the effluent was stored in the refrigerator until further use for the decolorization assay. The unsterilized effluent was immediately subjected to physico-chemical analysis using standard methods [6].

Spectral studies

The maximum absorbance of the filtered dye effluent was estimated using UV-Visible Spectrophotometer 108 (Systronics) and the λ_{max} was found to be at 645nm

Isolation and Screening of Dye Decolorizing Bacteria

Dye decolorizing bacteria were isolated from raw textile effluent collected from Karnataka Silk Industries Corporation, Mysore. The effluent sample was serially diluted with 9 ml of distilled water from 10^{-1} to 10^{-7} dilution. About 0.1 ml of the serially diluted sample of 10^{-5} and 10^{-7} dilution was poured onto the nutrient agar [(g/l) peptone - 5g, NaCl- 5g, yeast extract - 3g, agar-15g, pH -7] plates and spread evenly under the laminar air flow providing aseptic condition and incubated at 37ºC for 24 hrs. Individual colonies belonging to predominant types of microorganisms were purified by streaking (zigzag streaking) on to the same medium. By morphological colony characteristics, a total of 10 different predominant types of bacteria were identified and the approximate numbers of colonies were 376. By Gram's staining the purified isolates were examined microscopically to check their purity. Pure cultures obtained were maintained on nutrient agar at 4°C [7, 8 and 9].

The isolated pure colonies from the effluent were inoculated in 10 ml Nutrient broth (inoculum). Inoculum (2.5ml) was inoculated into 250 ml flasks containing 100 ml mineral salts medium and dye (0.01%). Mineral salts Basal medium had the following composition (g/l): Na_2HPO_4, 2.13; KH_2PO_4, 1.3; NH_4Cl, 0.5; $MgSO_4$, 0.2; tab water up to 1 litre and 1ml of trace element solution per litre. The trace element solution had the following composition (g/l): $MgSO_4.7H_2O$, 7.12; $ZnSO_4.7H_2O$, 0.044; $MnSO_4.4H_2O$, 0.081; $CuSO_4.5H_2O$, 0.0782; Na $_2MoO_4.2H_2O$, 0.025; FeSO4.7H2O, 0.498; Boric acid 0.1 and 0.27 ml of H_2SO_4. The final pH was adjusted to 7.0 using 0.1 NaOH or HCl as per requirement. The mineral salts medium was supplemented with 1.0 g/l yeast extract [10]. Each flask with 2.5 ml of inoculum was incubated on rotary incubator at 150 rpm for six days at room temperature for assessing the dye degrading ability. Among the 10 different predominant types of bacteria, one only was found to be a dye degrader. This strain with dye degrading potential was chosen and used to decolorize the textile dye effluent.

Molecular characterization of the bacterial strain

After purification by successive single colony isolation on an agar plate, the bacterial strain was identified by analysis of 16SrRNA sequences. The genomic DNA was extracted from the isolate by using the DNeasy kit (Qiagen). Two primers Forward = 5'-TGGTAGTCCACGCCCTAAC-3' and Reverse = 5' CTGGAAAGTTCCGTGGATGT-3' were applied for the amplification of the 16SrRNA gene. Polymerase chain reaction (PCR) was performed as follows: pre-

denaturation at 94°C for 5 min, 94°C for 30 s, 60°C for 30 s and 72°C for 45 s, 72°C for 2 min and hold at 4°C. Steps 2, 3 and 4 were repeated for 35 cycles. Nucleotide sequencing for these samples was performed using ABI 3500XL Genetic Analyzer at the Department of Studies in Biotechnology, University of Mysore, Mysore. The nucleotide sequences of the isolate obtained were compared to the sequences available in the public database using BLAST software (www.ncbi.nlm.nih.gov). Neighbor-joining method [11] was employed to construct the phylogenetic tree using MEGA4 software [12], and the maximum likelihood method was adopted for calculating the evolutionary distance.

Decolorization assay

Decolorization assay was carried out by taking 50 ml of the filtered, pre sterilized textile effluent sample with mineral salts basal media and the co-factors in 250 ml conical flasks. The resultant solution were subjected to two different culture conditions, one was the optimized condition, the values of which were obtained from optimization of direct dye Chrysophenine and Acid dye Red 3BN and the other unoptimized condition, which was the prevailing culture condition of the effluent. The resultant solution was inoculated as per the conditions specified in **Table 1.** All flasks were incubated for 24 hours, 5 ml aliquots were removed aseptically at an interval of 24 hours and subjected to centrifugation at 6000 rpm for 15 min. The supernatant was filtered using Sartorius filter disc. The optical density of the supernatant was measured at 645nm (Systronics Photoelectric Colorimeter 111). The percent decolorization was calculated using the following formula:

$$\% \text{ Decolorization} = \frac{\text{Initial optical density - Final optical density}}{\text{Initial Optical density}} \times 100$$

Characterization of textile dye effluent

The textile dye effluent was characterized before and after treatment by the bacterial isolate, *Shewanella putrefaciens* using standard methods [6]. Various physical and chemical parameters of the effluent were studied on the first day before treatment and 10^{th} day after treatment. Reductions of these parameters were assessed by the percent reduction on comparison with the raw untreated effluent using Eq.

$$A = 100(A_i - A_f)/A_i$$

Where A is the percent reduction of particular parameter, A_i initial concentration of same parameter and A_f the concentration of the same parameter after specified time [13]

RESULTS AND DISCUSSION

Identification of the bacterial isolate

The isolate used in this study was identified on the basis of 16SrRNA gene sequencing. The closest neighbor in GenBank database was found to *be S.putrefaciens* with the homology of 99.0%. The sequence was submitted to GenBank with an accession number of JN555612. The phylogenetic relationship of the isolate is shown in **Figure 1**

Decolorization of the textile effluent

The effluent was subjected to three culture conditions – one type of unoptimized and two types of optimized conditions as presented in **Table 2**. The effluent in the unoptimized condition showed 27.95% decolorization. This implies that, the dye house effluent as such under the prevailing conditions could not be decolorized efficiently. Therefore, it was essential to add some co-factors to the effluent, which would enhance the process of dye decolorization. Accordingly, the two optimized conditions – Chrysophenine and Red 3BN showed 63.15% and 89.4% decolorization of the effluent respectively. Since, Red 3BN optimized culture condition gave a maximum of 89.4% decolorization, it was further considered for physico-chemical analysis of the effluent after decolorization.

Table 1: Percentage decolorization of the effluent under optimized and unoptimized culture conditions

Culture condition	Constituents	Decolorization %
Unoptimized condition	pH – 8.2 Temperature - 37°C Inoculum size – 5% Volume of effluent – 50ml Incubation duration – 24 hr	27.95
Optimized condition	**1. Chrysophenine dye** pH – 4.39 Temperature - 32°C Inoculum size – 22.38% NH_4Cl – 0.26% Sucrose – 0.60% Starch – 0.20% Incubation duration – 24 hr	63.15
	2. Red 3BN dye pH – 8 Temperature - 30°C Inoculum size – 20.49% KH_2PO_4 – 0.35% Yeast extract – 0.34% $FeSO_4$ – 0.56% $MgSO_4$ – 0.32% NH_4Cl – 0.47% Sucrose – 0.86% Incubation duration – 24 hr	89.40

Table 2: Physico-chemical parameters of raw and treated dye house effluent

Parameter	Units	Raw Effluent	Treated effluent	% Reduction
pH	-	7.53	6.8	-
Temperature	(°C)	32.7	31.5	-
Hardness	mg/l	300	204	32.0
Calcium	mg/l	80	61.2	23.5
Magnesium	mg/l	14.4	12.24	15.0
Nitrate	mg/l	308	173	43.83
Sulphate	mg/l	34000	7000	79.4
Phosphate	mg/l	286	60.99	78.6
Total solids	mg/l	39640	30040	24.2
TSS	mg/l	80	36	55.0
Dissolved oxygen	mg/l	0.5	<0.1	-
Alkalinity	mg/l	309	224	27.5
COD	mg/l	1250	869	30.4
BOD	mg/l	680	400	41.1
Sodium	mg/l	7600	6300	17.1

Figure 1: Phylogenetic tree of bacterial strain *S.putrefaciens* based on 16SrRNA gene sequence

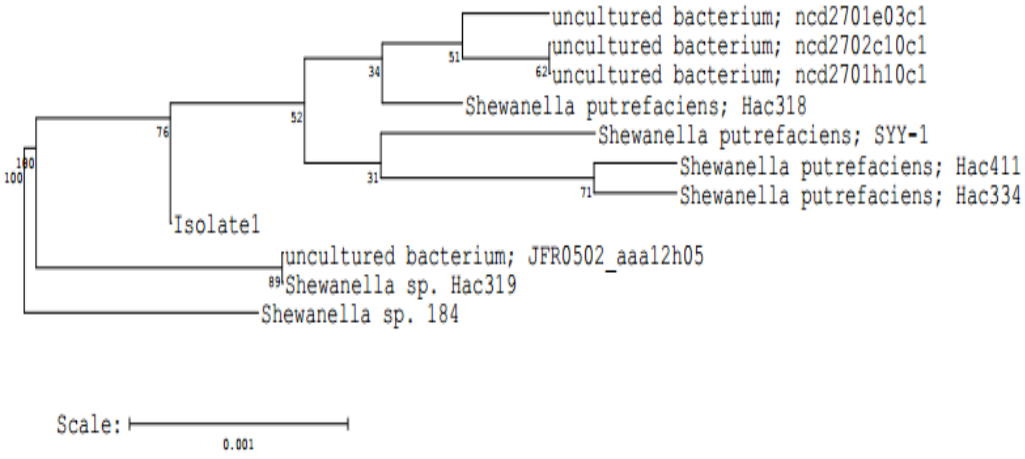

Figure 2: Effluent samples before and after treatment

Physicochemical Characterization of textile effluent

The effluent collected from textile industry in Nanjangud was purplish black with temperature of 32.7°C and slightly alkaline pH (7.53). Sulphates (34000 mg/L) and total solids (39640 mg/L) were quite high. Hardness, Calcium, Magnesium, Nitrate, Phosphate, Total Suspended Solids, dissolved oxygen and Alkalinity were 300 mg/l, 80 mg/l, 14.4 mg/l, 308 mg/l, 286 mg/l, 80 mg/l, 0.5, and 309 mg/l respectively. A high load of COD (1250 mg/l) and BOD (680 mg/l) were also observed. If this effluent is not treated before being discharged into the receiving river or soil, it can pose ecological threat. Considering this, the characterization of the effluent for the physicochemical parameters was done before and after treatment process. Comparison of chemical parameters between raw and treated effluent is shown in **Table 2.** **Fig 2** shows the effluent samples collected before and after decolorization process.

The results indicate that Hardness, Calcium, Magnesium, Nitrate, Sulphate, Phosphate, Total Solids, Total Suspended Solids, Dissolved Oxygen, Alkalinity, COD, BOD and Sodium showed considerable reduction after treatment. There was 79.4% reduction in Sulphate and 78.6% reduction in Phosphate after treatment. Sulphates may destroy fishes and microorganisms responsible for self purification of water. Impurities such as sulphates and nitrates can cause depletion of dissolved oxygen content of water [14].

Total suspended solids showed 55% reduction, followed by 43.83% in Nitrate, 41.1% in BOD, 32% in hardness and 30.4% in COD. The colloidal and suspended impurities cause turbidity in the receiving streams [15]. Total solids content was high which has great implications on the biological and physical waste water treatment processes [16, 17, and 18]. The dissolved minerals may increase salinity of the water and may render it unfit for irrigation. Previous study reported that high amount of total solids are one of the major sources of sediments, which reduce the light penetration and affect the photosynthesis, thereby decreasing purification by the microorganisms. The concentration of the solids in textile effluent is another matter of concern and it adds to the carcinogenic effect of the dyes [19].

The high BOD and COD values indicate that the effluent has high oxygen demanding waste [20] which cause the depletion of Dissolved oxygen content which is an essential requirement for aquatic life. The high COD value indicates the pollution potential of the textile industry effluent [21]. The results obtained after treatment indicate very good correlation with previous study [22] which reported the reduction in COD load of effluent below the upper limit of 25 mg/l. The COD was reduced from 1200 mg/l to 200 mg/l after 15 days of treatment. In the present study, after 10 days of incubation, the COD was reduced from 1250 mg/l to 869 mg/l. The high levels of COD in the textile effluent indicate the toxicity level of pollution [23] which is very harmful to entire ecology of aquatic

ecosystem of the receiving bodies. The reduction in COD and BOD after treatment with the bacterial strain was due to reduction of organic load from effluent and ultimately the toxicity [24]. The exceeded permissible limits of pollution parameters reduced the natural process of bioremediation. Totally, this indicates that the process of decolorization has also resulted in considerable reduction in the pollution load of the effluent and its discharge to the environment.

The results obtained clearly show that textile effluent is highly polluted and this is in close agreement with the previous studies [25, 26 & 15]. The removal efficiency of the physico-chemical parameters by *Shewanella putrefaciens suggested* that it could be used for bioremediation of industrial effluent.

CONCLUSION

The textile industry is discharging a high load of dyes and chemicals through effluent into the nearby outlet. *Shewanella putrefaciens* could be used for bioremediation of textile effluent. Significant reductions in some of the parameters were achieved with the isolate. Hence, the present work gives an indication that the bacterium, *Shewanella putrefaciens* can be used as an efficient biological tool for textile effluent treatment.

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